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Susceptibility of Adult Chickens, With and Without Prior Vaccination, to Challenge with Marek's Disease Virus

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SUMMARY. Marek's disease (MD) outbreaks can occur in previously healthy adult layer or breeder flocks. However, it is not clear whether such outbreaks are caused by recent challenge with highly virulent (vv and vv+) strains of MD virus (MDV; i. e., new infection hypothesis) or by exacerbation of an earlier MDV infection (i. e., old infection hypothesis). To discriminate between these hypotheses, adult White Leghorn chickens of laboratory strains or commercial crosses with or without prior vaccination or MDV exposure were challenged at 18–102 wk of age with highly virulent MDVs, and lesion responses were measured. Horizontal transmission was studied in one trial. Challenge of adult chickens, which were free from prior MDV vaccination or exposure, with highly virulent MDV strains induced transient paralysis or tumors in 60%–100% of 29 groups (mean = 91%), and horizontal spread of virus was detected. The magnitude of the response was similar to that induced by challenge at 3 wk of age. In contrast, comparable challenge of adult chickens, which had been vaccinated or exposed to MDV early in life, induced transient paralysis or tumors in 0%–6% of 12 groups (mean = 0. 5%), although some birds showed limited virologic evidence of infection and transmission of the virus to contacts. The MD responses were influenced by the virulence of the challenge virus strain, and to a lesser extent by virus dose and route of exposure. Strong inflammatory lesions were induced in the brain and nerves of adult specific pathogen-free (SPF) chickens at 9–15 days after infection. The low susceptibility of previously vaccinated and exposed groups to challenge at $\geq$18 wk of age suggests that late outbreaks of MD in commercial flocks are not likely a result of recent challenge alone and that additional factors could be involved.

RESUMEN. Susceptibilidad de aves adultas con y sin vacunación previa, a un desafío con el virus de la enfermedad de Marek.

Brotes de la enfermedad de Marek pueden ocurrir en parvadas de ponedoras o reproductoras adultas previamente sanas. Sin embargo, no esta claro si esos brotes son causados por un desafío reciente con cepas altamente virulentas (vv y vv +) del virus de la enfermedad de Marek (hipótesis de infección nueva) o por el contrario, son el resultado de un incremento de una infección temprana (hipótesis de infección previa). Con la finalidad de discernir entre estas dos hipótesis, se desafiaron a las 18 y 102 semanas de edad, aves leghorn blancas pertenecientes a líneas genéticas de laboratorio, con o sin vacunación o exposición previa al virus de la enfermedad de Marek, y se midió la respuesta de lesiones. En uno de los ensayos se estudió la transmisión horizontal. El desafío con una cepa altamente virulenta del virus de la enfermedad de Marek de aves sin vacunación o exposición previa al virus, produjo parálisis transitoria o tumores en el 60% al 100% de los 29 grupos (promedio = 91%), a su vez se detectó diseminación horizontal del virus. La magnitud de la respuesta fue similar a la inducida por el desafío a las tres semanas de edad. En contraste, el desafío de aves adultas vacunadas o expuestas temprano al virus de la enfermedad de Marek, indujo parálisis transitoria o tumores en el 0% al 6% de 12 grupos (promedio = 0.5%), sin embargo, algunas aves mostraron una evidencia virológica limitada de infección y de transmisión del virus a las aves contacto. Las respuestas a la enfermedad de Marek fueron influenciadas por la virulencia de la cepa de desafío y en menor proporción por la dosis del virus y la ruta de exposición. Entre 9–15 días posteriores a la infección se indujeron lesiones inflamatorias fuertes en el cerebro y nervios de las aves adultas. La baja susceptibilidad de los grupos vacunados o previamente expuestos al desafío a las 18 semanas sugiere que los brotes tardíos de la enfermedad de Marek en parvadas comerciales no son solamente el resultado de desafíos recientes y que factores adicionales pueden estar relacionados.

Key words: chicken, virus, Marek's disease, vaccine failure, age, adult

Abbreviations: ADOL = Avian Disease and Oncology Laboratory; AGP = agar gel precipitin; DPI = days postinoculation; FA = fluorescent antibody; HVT = turkey herpesvirus; IA = intra-abdominal; MD = Marek's disease; MDV = Marek's disease virus; PFU = plaque-forming units; SPF = specific pathogen free; SQ = subcutaneous; TP = transient paralysis; VE = virus exposed

Although perhaps best known as a disease of young chickens, Marek's disease (MD) tumors are also commonly seen in older chickens in commercial flocks. Clinical cases of MD usually appear before the onset of egg production and are characterized by variable mortality for a few weeks or throughout the productive life of the flock. In a few cases, MD first becomes obvious at older ages (>30 wk) and can even first occur after molting in preparation for a second lay cycle (25, 28, 48). Morrow and Fehler (30) indicate that MD outbreaks in adult chickens have become very common. Such late outbreaks can be important causes of economic loss, but the responsible mechanism is poorly understood. One theory is that adult chickens are susceptible to new infection, which cycles in the flock and induces tumors. Another theory is that environmental factors exacerbate preexisting infections, resulting in the onset of tumors in adult birds.

When evaluating the susceptibility of adult chickens to challenge with Marek's disease virus (MDV) in the laboratory, it is necessary to consider prior exposure to MD vaccines or MDV field strains. Chickens without prior MDV exposure or vaccination are designated here as specific pathogen free (SPF). Chickens with prior MD vaccination or prior exposure to MDV field strains are designated as virus exposed (VE). SPF and VE chickens can be defined further by the absence or presence, respectively, of MDV antibodies. Virtually all chickens in commercial environments are of the VE class, although specific MD vaccine strains and the intensity of subsequent field exposure will vary among flocks. Challenge experiments conducted in laboratory environments have used chickens of both types.

Most prior laboratory studies on challenge of older chickens have used SPF chickens and low-virulence MDV strains. So-called age

resistance is considered an expression of genetic resistance and is best demonstrated in SPF chickens of resistant genotypes (8, 9, 42). Early studies in SPF chickens of varying genotypes have usually resulted in a poor tumor response when chickens were challenged at 12–22 wk of age (8, 42, 53), but in one exceptional study, 60%–80% of Athens-Canadian SPF chickens exposed to the GA strain at 15 wk of age developed MD (1). The susceptibility of SPF chickens subjected to natural challenge by placement on an infected commercial farm at various ages declined with age; exposures of up to 12 wk of age resulted in 42%–45% MD and exposures at 13–20 wk and 33–55 wk resulted in 8% and 6% MD, respectively (44). Sharma *et al.* (42) observed that after challenge of 12-wk-old chickens, MD lesions were induced but tended to regress, thus establishing a possible mechanism for the observed resistance. Thus, challenge of adult SPF chickens with low-virulence strains has usually, but not always, resulted in a low or variable frequency of tumor responses. Furthermore, transient paralysis (TP) or other neurologic signs were not reported in any of these studies. More recently, Rosenberger *et al.* (37) reported that exposure of adult SPF chickens to high-virulence strains appeared to induce high rates of lymphomas and neurologic disease, but few details were provided.

Few experimental studies with VE chickens have been reported. Ianconescu *et al.* (20) removed nonvaccinated chickens from a commercial farm environment at 5, 7, and 12 wk of age and placed them in laboratory isolators in which MD tumor responses to inoculation and contact challenge with the low-virulence JM strain were determined. The nonvaccinated, farm-reared chickens were exposed to MD on the farm because the uninoculated controls developed MD tumors and antibodies when placed in isolators. At 12 wk, the farm-reared chickens appeared totally refractory to JM challenge because the tumor incidence in challenged and nonchallenged birds was identical (10%–12%) (20). The role of natural vaccination by avirulent strains (4) probably contributes to the relative resistance of older VE chickens to MDV challenge in the field. MD-vaccinated chickens are also VE, and their response to challenge at different ages has been the subject of many studies, the results of which depend largely on the efficacy of the specific vaccine tested and the virulence of the challenge. In one study, day-old turkey herpesvirus (HVT) vaccination was fully effective when challenge with the low-virulence JM strain was delayed until 40 wk, although the gross tumor response of the nonvaccinated controls was so low that the responses had to be measured by a short-term histologic assay (51). These results are consistent with very early reports (22, 23) that adult chickens (presumably VE) were resistant when exposed to MD (cited by Biggs (3)). Thus, older VE chickens appear highly resistant to challenge with low-virulence MDV strains. However, data on challenge of adult VE chickens with highly virulent strains are lacking.

Virologic responses are also influenced by age at exposure. Recent studies showed that in older SPF chickens, cytolytic infections were resolved more rapidly (7) and virus load is somewhat lower (11). Little information is available on the influence of age on induction of TP although in one study, 18-wk-old SPF chickens were susceptible to acute TP when inoculated with high doses of MDV strain 584A (very virulent plus [vv+] pathotype) (49).

The purpose of this study was to test the susceptibility of adult VE chickens of susceptible laboratory strains and more resistant commercial stocks to challenge with highly virulent MDVs. Studies on SPF chickens without prior exposure or vaccination and lacking MD-related antibodies were also performed. Chickens were exposed by artificial and natural routes to varying doses of MDV strains representing v (virulent), vv (very virulent), and vv+ (very virulent plus) pathotypes (47). Exposed chickens were evaluated for clinical signs of TP and for MD lymphoma development. In one trial, transmission of virus from inoculated older chickens to uninoculated age-matched contact birds was measured. Results were analyzed to determine the likelihood that MD outbreaks in adult chickens result directly from recent virus exposure.

MATERIALS AND METHODS

Chickens. Chickens from the Avian Disease and Oncology Laboratory (ADOL) breeding flock included the 15 × 7 cross, which were progeny of inbred line $15I_5$ males and line 7_1 females and were considered highly susceptible to MD lymphomas. Chickens of inbred line 7_2 and line 7_1 were also used. White Leghorn chickens representing three commercial egg-laying crosses from two companies were obtained and designated as Com-A, Com-B, and Com-C, respectively.

For experiments in which chickens were challenged at 18 wk, 15 × 7 chickens were hatched at ADOL and reared in plastic canopy isolators until the time of challenge. For experiments in which chickens were used at >18 wk, 7_2 or line 7_1 chickens were obtained as adults from the ADOL SPF breeding flock (nonvaccinated and isolator reared) or the ADOL antibody-positive breeding flock (vaccinated and pen reared). The SPF breeding flock received no MD vaccinations or MDV exposure and was negative for antibodies to MDV on routine surveillance tests. The antibody-positive flock was vaccinated at hatch with 2000 plaque-forming units (PFU) of HVT and at about 25 wk with 2000 PFU of SB-1 and Md11/75C viruses to ensure exposure to all three viral serotypes. ADOL breeding flocks were also negative for exogenous avian leukosis virus and reticuloendotheliosis virus on the basis of periodic serology. The status for chicken infectious anemia virus was not determined. All animal experiments were approved by the ADOL Institutional Animal Care and Use Committee.

Viruses. The serotype 1 MDV strains used included several representatives of each pathotype to facilitate comparisons. The vv+ strains were 584A, 645, 648A, and 652 (47); the vv strains were Md5 (52), 595 (47), and 549A (47); and the v strains were JM/102W (41, 43), GA/22 (12, 36), 617A (47), and RB1B (40). The ADOL preparation of RB1B was assigned to pathotype v on the basis of pathotyping tests (16) and appeared to be less virulent than the parent strain. All serotype 1 strains were at relatively low (6–18) cell culture passage and consisted of suspensions of infected duck embryo fibroblast cultures that had been cryopreserved and stored at −196 C. The pathotypes of the serotype 1 strains are designated as v, vv, and vv+ according to the classification by Witter (47). The serotype 1 strains have also been classified according to neuropathotype: type A included strain JM/102W and RB1B; type B included strains Md5, GA/22, and 617A; and type C included strains 584A, 648A, 652, 595, and 549A (15). Avirulent vaccine strains included the FC126 strain of HVT (serotype 3) (31, 50), SB-1 (serotype 2) (39), and attenuated Md11/75C (serotype 1) (45). Virus stocks were free of contamination with avian leukosis, reticuloendotheliosis, and chicken anemia viruses.

Virus and antibody assay. Virus isolation for serotype 1 MDV was conducted in duck embryo fibroblast monolayer cultures inoculated with 10^6 buffy coat cells and observed for 7–9 days for plaque formation (46). Agar gel precipitin (AGP) tests with the use of an antigen prepared from the feather tips of chickens challenged with serotype 1 MDV were performed on sera or plasma to detect MD antibody in SPF chickens before challenge (10). Fluorescent antibody (FA) tests were conducted on infected duck embryo fibroblast cultures by an indirect staining method (35) with the monoclonal antibody H19 (27), which is specific for pp38 antigen of serotype 1 MDV.

Experimental design. Chickens destined for adult challenge and long-term holding in isolators were detoed and dubbed at hatch. SPF chickens received no vaccinations, were reared in isolators from the day of hatch, and were monitored in most cases for MD antibody by AGP tests before challenge as adults to confirm their SPF status. For trials 2 and 4, VE chickens were vaccinated at hatch with 2000 PFU of HVT and were additionally exposed at 5 wk of age to 500 PFU of the low-virulence JM/102W strain of MDV. For trial 5, VE chickens were

obtained from the ADOL antibody-positive breeding flock. In all experiments with adult chickens, males were removed as soon as secondary sexual characteristics were apparent, and only females were retained to provide better husbandry for extended holding periods. Inoculation of both young and adult chickens was by the intra-abdominal (IA) or, in one case, by the subcutaneous (SQ) route with a volume of 0.1 ml. Adult chickens were typically debeaked at the time of inoculation unless this had been done earlier. Experiments were terminated 8–12 wk postinoculation.

Necropsies were performed on dead birds during the experiment and on all birds killed at the end. Clinical neurologic signs were recorded daily in some trials to detect TP. The criteria for scoring TP clinical signs and observational techniques are provided elsewhere (16). Birds were considered positive for TP signs (clinical TP) when flaccid paralysis of the neck, wings, or legs was noted on one or more days during the observation period. Birds were considered positive for TP death (acute TP) when death occurred from 8 to 18 days postinoculation (DPI), whether or not prior paralysis was observed. Birds were considered positive for MD when nerve enlargements or visceral lymphomas were observed on gross necropsy examination and were recorded as MD death or total MD (includes MD-positive birds killed at the end of the trial). Histologic examination of sections stained with hematoxylin and eosin was used to resolve questionable gross lesions and to document unique pathology. Birds that died after 18 days but lacked gross lesions of MD were considered to be nonspecific (ns death). Nonresponders were the birds that did not develop TP signs, did not die of TP or MD, and lacked MD-related gross lesions at termination (in other words, ns deaths without prior TP clinical signs plus survivors without MD lesions or prior TP clinical signs). Total MD–related response was calculated as 100 minus the percent nonresponders. In these studies, the number of birds at risk (*n*) was the number of chickens alive at day 8.

Trial 1A consisted of four female chickens of SPF line 7_2, obtained from the laboratory SPF breeding flock, that were inoculated at 30 wk with 10,000 PFU of strain 584A or 645 and housed in modified Horsfall–Bauer isolators, ostensibly to produce stocks of antiserum. In trial 1B, SPF female chickens of line 7_1, obtained from the laboratory SPF breeding flock, were inoculated at 102 wk with 500 PFU of strains 648A, Md5, and JM/102W. Observations for clinical TP were conducted 8–13 DPI.

In trial 2, female SPF chickens of line 15 × 7 were reared from hatch in plastic canopy isolators and were challenged at 18 wk with three vv+ strains (645, 648A, and 652) and three vv strains (549A, 595, and Md5) of MDV by three methods: IA inoculation with 10,000 PFU, IA inoculation with 500 PFU, and contact exposure (series A, B, and D). The contact exposure was accomplished by inserting into a small wire enclosure within the recipient isolator 6 line 15 × 7 chickens that were 4 wk of age and had been inoculated 2 wk earlier with 500 PFU of the appropriate virus strain. The donor chickens were removed at 6 wk of age to provide a 2-wk exposure period. In series C, male and female SPF chickens of line 15 × 7 were challenged by IA inoculation at 3 wk. In series E, female VE chickens of line 15 × 7 were challenged by contact exposure at 18 wk. Clinical signs were not observed. The five series were done at different times for logistic reasons but were otherwise comparable and are reported together.

Trial 3 was similar to trial 2 except that one additional vv+ strain (584A) and four v strains (JM/102W, GA/22, 617A, and RB1B) were used to challenge female 15 × 7 chickens at 18 wk. Observations for clinical TP were conducted from 8 to 18 DPI.

Trial 4 was designed to test the susceptibility of female SPF and VE chickens of three commercial strains (Com-A, Com-B, and Com-C) at 18 wk. Line 15 × 7 chickens were used as a control. Fertile eggs were received from the respective companies; incubation and hatching was done at ADOL. Each chicken strain was subdivided into three lots, two of which were maintained as SPF and one of which was reared as VE. All chickens were reared in flexible canopy isolators from hatch. Males were discarded at 4 wk. At 18 wk, one group of SPF and one group of VE female chickens of each strain was challenged by contact exposure to vv+ strain 648A. The method of contact exposure was as described for trial 2, except that eight donor chicks were used in each isolator and the exposure duration was 4 wk. A third group of female chickens of each strain was kept as unchallenged controls. Observations for clinical TP were conducted from 8 to 18 DPI.

Trial 5 examined horizontal transmission after exposure of adult SPF and VE chickens. The main questions were whether adult VE chickens would transmit virus after exposure to vv+ MDV strains and whether such transmission would cause disease in other age-matched adult VE chickens. SPF chickens were used as controls. Female chickens of line 7_1 were obtained from either the ADOL SPF breeder flock or the age-matched ADOL antibody-positive breeder flock (VE). The chickens were placed in large, negative pressure isolators and acclimatized for 2 wk and were 81 wk of age at the start of the trial. Because each isolator could only hold six adult chickens, four isolators were used for each treatment with two birds per treatment per isolator. Thus one treatment consisted of four isolators, each containing two VE chickens inoculated with 2500 PFU of MDV strain 648A (vv+) and two uninoculated SPF and two uninoculated VE chickens. In a second treatment, four isolators each contained two SPF chickens inoculated and reared with two uninoculated SPF and two uninoculated VE chickens. Uninoculated SPF and VE controls were also maintained in separate isolators. One group of day-old 15 × 7 chickens was challenged with 2500 PFU of 648A to confirm potency of the inoculum. To ensure that all inoculated chickens received the virus, the inoculum was divided and half was given by IA and SQ routes, respectively. Clinical neurologic signs were observed from 9 to 44 DPI. Blood was collected for viremia at 0, 4, and 12 wk. Virus plaques in some cell cultures were stained with monoclonal antibody H19 with an indirect FA assay to distinguish serotype 1 virus. Antibody tests were done by AGP tests on plasma collected at 0 and 12 wk. All chickens were killed at 12 wk (93 wk of age) and examined for gross lesions.

Pathology. Brains, peripheral nerves, and selected other tissues from a few representative SPF chickens derived from trials 1A, 2, and 5 were examined histologically. One set of samples represented 16 chickens that were killed 8–15 DPI with clinical signs of TP. A second set of samples represented 20 chickens that died or were killed at 29–98 DPI with gross or suspicious MD lesions; some of these birds (trial 5) had also experienced a prolonged period of ataxia. The chickens were inoculated or exposed at 18, 30, or 81 wk of age to various MDV strains representing vv or vv+ pathotypes (except as otherwise noted).

RESULTS

Preliminary observations. Two preliminary trials provided data on the susceptibility of SPF chickens to MDV challenge (Table 1). In trial 1A, 30-wk SPF chickens were challenged with high doses of vv+ strains 584A and 645. Two birds were inoculated in each lot. Unexpectedly, all four birds became moribund with acute TP and were killed at 9 DPI; no gross lesions were noted at necropsy. In trial 1B, 102-wk SPF chickens were inoculated with 500 PFU of three MDV strains representing all three pathotypes. Strain 648A (vv+ pathotype) induced TP signs in 10 of 12 chickens, eight of which died before day 18. Strain Md5 (vv pathotype) induced TP signs or MD lesions in 9 of 12 chickens. Of 12 chickens inoculated with strain JM/102W (v pathotype), none developed TP signs and only two developed MD nerve lesions. No TP or MD occurred in control birds, but the rate of nonspecific mortality was relatively high in all lots. These data indicate a high susceptibility of adult SPF chickens to challenge with highly virulent MDV and a relationship of viral pathotype to disease response.

Effect of virus strain and dose. Results of trial 2 are presented in Tables 2 and 3. SPF female chickens challenged with six highly virulent MDV strains at 18 wk by three methods were highly susceptible to the induction of TP and MD lesions (see series A, B, and D). The total MD–related response rates in 18 exposed groups were 83%–100%. Frequencies of TP deaths were generally highest in the contact-exposed group, followed by the low-dose (500 PFU) and high-dose (10,000 PFU) groups, respectively. Higher TP

Table 1. Responses induced in adult SPF hens inoculated with Marek's disease virus (trials 1A and B).

			Exposure class		MDV challenge					% Disease response (through 57 DPI)[C]						Median days to death	
Trial	Chicken strain	Challenge age (wk)	Type	Prechallenge antibody[A]	Virus strain	Pathotype	Route	Dose (PFU)	n[B]	TP signs	TP death	MD death	ns death	Total MD	Non-responders	TP	MD
1A	7_2	30	SPF	NT	584A	vv+	IA	10,000	2	100	100	0	0	0	0	9[D]	
		30	SPF	NT	645	vv+	IA	10,000	2	100	100	0	0	0	0	9[D]	
1B	7_1	102	SPF	NT	648A	vv+	IA	500	12	83	67	0	33	0	17	12	
		102	SPF	NT	Md5	vv	IA	500	12	42	8	8	50	33	25	15	39
		102	SPF	NT	JM/102W	v	IA	500	12	0	0	0	25	17	83		
		102	SPF	NT	None			0	11	0	0	0	18	0	100		

[A]NT = not tested but presumed negative.
[B]n = birds alive at 8 days.
[C]Response categories explained in Materials and Methods.
[D]Birds killed when moribund.

responses tended to be associated with shorter median days to death. Approximately 50% of birds survived mortality from TP, and of these, 86% developed MD lesions. Consistently robust TP and MD responses were induced by the three vv+ strains. Responses induced by the three vv strains were more variable; although strain 549A was similar to the vv+ strains, strain 595 was noticeably weaker, and strain Md5 (neuropathotype B) was weakest, at least when measured by induction of acute TP. However, when total MD–related responses were considered, there was no difference between vv+ and vv viral strains.

The 3-wk SPF chickens (series C) challenged with 500 PFU of the respective viral strains developed slightly weaker total MD–related responses (mean = 76%) than counterpart groups in series B (mean = 96%; Table 2). Responses induced by the vv+ strain 652 were unexpectedly low (three of 12 birds with clinical TP, no MD lesions).

In series E, the susceptibility of VE class chickens to MDV challenge was examined (Table 3). Contact challenge of these chickens with six different MDV strains induced no TP deaths and no MD gross lesions in any bird. Virus isolation was performed on buffy coat cells from five chickens per treatment at termination to see whether serotype 1 virus was present. Although virus was isolated from 12 of 30 chickens, none of these isolates stained with serotype 1–specific H19 antibody (data not shown) and thus were assumed to be HVT. Because chickens in series D developed a robust response to similar challenge exposure, it seems probable that the challenge in series E was sufficient but failed to induce either viremias or lesions.

Trial 3 was designed to determine the lesion response of 18-wk female SPF birds to challenge with five additional MDV strains (four v pathotypes and one vv+ pathotype) at two dose levels. None of the low-virulence strains induced TP death at either dose and most induced no clinical TP, although RB1B induced 21% clinical TP at the 10,000 PFU dose. Strain JM/102W induced high rates (79%–100 %) of MD lesions at both doses. All positive birds had enlarged nerves, none had visceral tumors, and none died before termination

Table 2. Influence of virus strain, dose and age at inoculation on responses induced in adult hens inoculated with Marek's disease virus (trial 2).

			Exposure class		MDV challenge					% Disease response (through 60–64 DPI)[B]					Median days to death	
Series	Chicken strain	Challenge age (wk)	Type	Prechallenge antibody[A]	Virus strain	Pathotype	Route	Dose (PFU)	n	TP death	MD death	ns death	Total MD	Non-responders	TP	MD
A	15 × 7	18	SPF	0/3	645	vv+	IA	10,000	13	31	69	0	69	0	11	33
		18	SPF	0/3	648A	vv+	IA	10,000	12	33	50	17	50	17	14	35
		18	SPF	0/3	652	vv+	IA	10,000	13	8	77	15	77	15	13	33
		18	SPF	0/3	549A	vv	IA	10,000	8	38	63	0	63	0	13	51
		18	SPF	0/3	595	vv	IA	10,000	15	20	47	13	67	13	15	48
		18	SPF	0/3	Md5	vv	IA	10,000	12	0	18	0	91	9		46
		18	SPF	0/3	None			0	13	0	0	0	0	100		
B	15 × 7	18	SPF	0/3	645	vv+	IA	500	12	67	25	8	25	8	13	34
		18	SPF	0/3	648A	vv+	IA	500	12	75	25	0	25	0	12	32
		18	SPF	0/3	652	vv+	IA	500	12	67	33	0	33	0	12	32
		18	SPF	0/3	549A	vv	IA	500	12	50	33	8	42	8	12	50
		18	SPF	0/3	595	vv	IA	500	12	33	42	0	58	9	13	52
		18	SPF	0/3	Md5	vv	IA	500	12	0	8	0	100	0		43
		18	SPF	0/3	None			0	11	0	0	0	0	100		
C	15 × 7	3	SPF	NT	645	vv+	IA	500	12	25	50	25	50	25	11	41
		3	SPF	NT	648A	vv+	IA	500	12	33	50	17	50	17	11	31
		3	SPF	NT	652	vv+	IA	500	12	33	0	0	0	67	11	
		3	SPF	NT	549A	vv	IA	500	12	67	17	17	17	17	11	42
		3	SPF	NT	595	vv	IA	500	12	33	17	8	50	17	11	49
		3	SPF	NT	Md5	vv	IA	500	12	0	0	0	100	0		
		3	SPF	NT	None			0	12	0	0	0	0	100		

[A]Samples positive by AGP tests/total samples tested. NT = not tested but presumed negative.
[B]Response categories explained in Materials and Methods.

Table 3. Influence of prior exposure on responses induced in adult hens exposed by contact to Marek's disease virus (trial 2 continued).

Series	Chicken strain	Challenge age (wk)	Exposure class: Type	Exposure class: Prechallenge antibody[A]	MDV challenge: Virus strain	MDV challenge: Pathotype	MDV challenge: Route	*n*	% Disease response (through 65–68 DPI)[B]: TP death	MD death	ns death	Total MD	Non-responders	Median days to death: TP	Median days to death: MD
D	15 × 7	18	SPF	0/3	645	vv+	Contact	18	100	0	0	0	0	11	
		18	SPF	0/3	648A	vv+	Contact	19	90	5	5	5	0	13	33
		18	SPF	0/3	652	vv+	Contact	17	88	12	0	12	0	11	39
		18	SPF	0/3	549A	vv	Contact	18	94	0	6	0	0	12	
		18	SPF	0/3	595	vv	Contact	19	53	16	0	47	0	14	52
		18	SPF	0/3	Md5	vv	Contact	19	26	21	0	74	0	16	61
		18	SPF	0/3	None			20	0	0	0	0	100		
E	15 × 7	18	VE[C]	3/3	645	vv+	Contact	18	0	0	0	0	100		
		18	VE[C]	3/3	648A	vv+	Contact	19	0	0	0	0	100		
		18	VE[C]	3/3	652	vv+	Contact	17	0	0	0	0	100		
		18	VE[C]	3/3	549A	vv	Contact	17	0	0	0	0	100		
		18	VE[C]	3/3	595	vv	Contact	20	0	0	0	0	100		
		18	VE[C]	3/3	Md5	vv	Contact	19	0	0	0	0	100		
		18	VE[C]	3/3	None			18	0	0	0	0	100		

[A]Samples positive by AGP tests/total samples tested.
[B]Response categories explained in Materials and Methods.
[C]VE = 2000 PFU of HVT at hatch (IA inoculation) plus 500 PFU of JM/102W at 5 wk (IA inoculation).

of the trial. This response was greater than that previously obtained with JM/102W virus in older 7_1 chickens (Table 1). In contrast, the highly virulent 584A strain induced TP and MD lesion responses in nearly all inoculated chickens at both doses (Table 4).

Susceptibility of commercial strains. SPF female chickens of all three commercial White Leghorn strains were susceptible to contact challenge at 18 wk with the vv+ 648A strain (Table 5). Overall, the percentage of chickens that developed MD-related responses ranged from 60% to 75%. The same contact challenge induced clinical signs or MD lesions in 95% of female line 15 × 7 SPF controls. Nonspecific deaths were high in SPF contact-challenged groups of all three commercial strains. No MD occurred in the nonchallenged SPF controls.

In contrast, none of the female VE chickens of three commercial strains or the 15 × 7 controls developed TP, and only a single bird of the Com-C strain developed gross MD lesions after contact challenge at 18 wk, indicating that VE chickens in this trial were highly resistant to MD challenge. The one MD-positive chicken in the VE Com-C group died with a gonad tumor but no nerve lesions at 30 days postchallenge. Because two additional chickens of this group died with MD tumors before 648A challenge, presumably because of the 5-wk JM/102W exposure (Table 5), it is not clear whether the tumor in this one chicken was induced by the 648A challenge.

Virus transmission in adult chickens. Data are presented sequentially on inoculated SPF chickens, chickens in contact with inoculated SPF chickens, inoculated VE chickens, chickens in contact with inoculated VE chickens, and control groups (Tables 6, 7).

Inoculated SPF chickens (housed in isolators 1–4) developed a strong MD response. Of the 10 birds, all showed clinical TP and nine either died from acute TP or had gross nerve lesions at 27–40 DPI after a period of persistent ataxia (Tables 6, 7). No visceral lymphomas were noted. Serotype 1 virus was isolated from two of two chickens at 4 wk (Table 6). In isolators 1 and 3, both of the original inoculated SPF birds died early from acute TP. In these isolators, one additional inoculated SPF bird (from a stock of extra age-matched inoculated birds held separately for this purpose) was

Table 4. Responses induced in adult SPF hens inoculated with additional strains of Marek's disease virus (trial 3).

Group	Chicken strain	Challenge age (wk)	Exposure class: Type	Exposure class: Prechallenge antibody[A]	MDV challenge: Virus strain	MDV challenge: Pathotype	MDV challenge: Route	MDV challenge: Dose (PFU)	*n*	% Disease response (through 63 DPI)[B]: TP signs	TP death	MD death	ns death	Total MD	Non-responders	Median days to death: TP	Median days to death: MD
A	15 × 7	18	SPF	0/1	JM/102W	v	IA	10,000	14	0	0	0	0	79	21		
		18	SPF	0/2	GA/22	v	IA	10,000	14	0	0	0	0	0	100		
		18	SPF	0/5	617A	v	IA	10,000	12	0	0	0	0	0	100		
		18	SPF	0/3	RB1B	v	IA	10,000	14	21	0	0	0	7	71		
		18	SPF	0/3	584A	vv+	IA	10,000	14	93	64	0	7	21	7	9	
B	15 × 7	18	SPF	0/1	JM/102W	v	IA	500	14	0	0	0	0	100	0		
		18	SPF	0/3	GA/22	v	IA	500	14	0	0	0	0	0	100		
		18	SPF	0/3	617A	v	IA	500	14	0	0	0	0	0	100		
		18	SPF	0/4	RB1B	v	IA	500	13	0	0	15	0	15	85		45
		18	SPF	0/3	584A	vv+	IA	500	14	43	14	0	14	71	0	15	
C	15 × 7	18	SPF	0/1	None			0	14	0	0	0	0	0	100		

[A]Samples positive by AGP tests/total samples tested.
[B]Response categories explained in Materials and Methods.

Table 5. MD susceptibility of adult vaccinated and nonvaccinated chickens of three commercial lines (trial 4).

Chicken Strain	Challenge age (wk)	Exposure class		MDV challenge			% Disease response (through 69 DPI)[B]						Median days to death	
		Type	Prechallenge antibody[A]	Virus strain	Route	*n*	TP signs	TP death	MD death	ns death	Total MD	Non-responders	TP	MD
Com-A	18	SPF	0/3	648A	Contact	20	55	10	30	60	30	25	15	35
		VE[C]	2/3	648A	Contact	20	0	0	0	5	0	100		
		SPF	0/3	None		21	0	0	0	0	0	100		
Com-B	18	SPF	0/3	648A	Contact	20	25	0	40	60	40	40		38
		VE[C]	3/3	648A	Contact	20	0	0	0	0	0	100		
		SPF	0/3	None		21	0	0	0	10	0	100		
Com-C	18	SPF	0/3	648A	Contact	20	20	5	40	35	45	40	16	49
		VE[C]	3/3	648A	Contact	18[D]	0	0	6	0	6	94		30
		SPF	0/3	None		20	0	0	0	0	0	100		
15 × 7	18	SPF	0/3	648A	Contact	20	95	90	0	10	0	5	15	
		VE[C]	3/3	648A	Contact	20	0	0	0	0	0	100		
		SPF	0/3	None		20	0	0	0	0	0	100		

[A]Samples positive by AGP tests/total samples tested.
[B]Response categories explained in Materials and Methods.
[C]Vaccinated with 2000 PFU of HVT at hatch, inoculated with 500 PFU of JM/102W at 5 wk.
[D]Two birds died with MD tumors at days 83 and 108 before contact challenge with strain 648A at day 126 but subsequent to JM/102W exposure at day 36 (5 wk).

introduced to each isolator on 13 DPI to ensure continuity of exposure to other groups (Table 7).

Transmission from inoculated SPF chickens to SPF contacts was robust. Of the eight contact-exposed SPF birds (also housed in isolators 1–4), all developed clinical TP and six died with acute TP; one surviving bird developed enlarged nerves (Tables 6, 7). The time of onset of clinical TP varied from 24 to 32 days postexposure among different isolators but tended to be consistent between the two SPF contact birds within an isolator (see isolators 2, 3, and 4; Table 7). Because the latent period to clinical TP induction in inoculated adult SPF chickens was about 10 days in this study (Table 7), the time of effective transmission from adult donors to recipients appeared to vary from about 14 DPI (isolator two) to 22 DPI (isolator four). In contrast, none of the contact-exposed VE pen mates developed clinical signs or lesions, although this group could have been infected because serotype 1 virus was isolated from one of eight chickens at 12 wk and no isolations were made at any time from noninoculated VE control chickens (Table 6).

Inoculated VE chickens (housed in isolators 5–8) were highly resistant to induction of clinical disease. None of the eight chickens developed TP, and no MD tumors or nerve lesions were observed (Table 6). However, serotype 1 MDV was isolated from one of three birds at 4 wk and from none of seven birds at 12 wk.

Transmission from inoculated VE chickens to contacts appeared to be minimal. Of the eight contact-exposed SPF pen mates (also housed in isolators 5–8), no clinical TP or MD lesions were observed and no serotype 1 virus was isolated, but transmission was indicated by the presence of antibody in four of six birds at 12 wk (Table 6). None of the contact-exposed VE pen mates developed signs, lesions, or virologic evidence of infection.

All uninoculated controls (housed in isolators 9 and 10) were free of lesions and serotype 1 virus, including a group of SPF

Table 6. Responses induced in SPF and VE adult hens of line 7_1 by inoculation and contact exposure (trial 5).

Isolators	Chicken strain	Challenge age	Exposure class		MDV challenge				% Disease response (through 98 DPI)[A]						Virologic response[B]			
			Type	Prechallenge antibody	Virus strain	Route	Dose (PFU)	*n*	TP signs	TP death	MD death	ns death	Total MD	Non-responders	Virus isolated (wk DPI) 0	4	12	Antibody (12 wk DPI)
1–4[C]	7_1	81 wk	SPF	0/8[D]	648A	IA + SQ	2500	10	100	50	40	10	40	0	0/8	2/2		
		81 wk	SPF	0/8	648A	Contact		8	100	75	12	12	12	0	0/8	4/6	0/1	0/1[D]
		81 wk	VE[E]	8/8	648A	Contact		8	0	0	0	0	0	100	0/7	0/3	1/8	
5–8[C]	7_1	81 wk	VE[E]	8/8	648A	IA + SQ	2500	8	0	0	0	12	0	100	0/4	1/3	0/7	
		81 wk	SPF	0/8	648A	Contact		8	0	0	0	25	0	100	0/8	0/8	0/6	4/6
		81 wk	VE[E]	8/8	648A	Contact		8	0	0	0	0	0	100	0/8	0/4	0/8	
9	7_1	81 wk	VE[E]	3/3	None	Contact	0	3	0	0	0	0	0	100	0/2	0/1	0/3	
		81 wk	SPF	0/3	None	Contact		3	0	0	0	0	0	100	0/3	0/3	0/3	0/3
10	7_1	81 wk	SPF	0/3	None	Contact	0	3	0	0	0	33	0	100	0/3	0/3	0/2	0/2
HB6	15×7	1 day	SPF	ND	648A	IA	2500	12	ND	8	83	8	83	9	ND	ND	ND	ND

[A]Response categories explained in Materials and Methods.
[B]MDV isolations = number of serotype 1 MDV isolates/total samples tested. Antibody = no. positive by AGP test/total samples tested (from lots lacking antibody at start of trial).
[C]Data pooled from similarly treated chickens in each of four replicate isolators.
[D]Samples positive by AGP tests/total samples tested.
[E]HVT vaccine inoculated at hatch; SB-1 and Md11/75C vaccines inoculated at 25 wk.

Table 7. Temporal pattern of clinical neurologic signs and MD nerve lesions in inoculated SPF chickens and their contact groups (trial 5).[A]

MDV challenge	Prior exposure type	Isolator[B]	Band	Neurologic signs (DPI) 9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	Nerve lesions at death
648A[C]	SPF	1	K1423	—	P	P	D																																	
	SPF	1	K1424	P	P	P	D																																	
	SPF	1	K1460[D]					P	A	A	A	A	A	A	A	A	A	A	A	A	A	D																	Gross pos
	SPF	2	K1429	—	P	P	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D															Gross pos
	SPF	2	K1430	—	P	P	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D									Gross pos
	SPF	3	K1435	—	P	P	P	D																															
	SPF	3	K1436	—	P	D																																	
	SPF	3	K1457[D]					A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D				Gross pos
	SPF	4	K1441	P	P	P	D																																
	SPF	4	K1442	—	—	P	A	A	—	—	—	—	—	—	A	A	A	A	A	A	A	A	A	A	A	D													Gross neg[E]
Contact	SPF	1	K1425	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	A	—	—	—	—	—	—	—	—	—	—	—	—	—	
	SPF	1	K1426	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	D								
	SPF	2	K1431	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	P	P	D																	
	SPF	2	K1432	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	P	P	D																	
	SPF	3	K1437	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	P	D															
	SPF	3	K1438	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	P	P	D														
	SPF	4	K1443	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	P	D										
	SPF	4	K1444	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	P	P	P	A	—	—	—	—	—	—	—	Gross pos
Contact	VE[F]	1	K1427	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	VE[F]	1	K1428	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	VE[F]	2	K1433	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	VE[F]	2	K1434	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	VE[F]	3	K1439	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	VE[F]	3	K1440	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	VE[F]	4	K1445	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	VE[F]	4	K1446	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

[A]Abbreviations: P = paralysis; A = ataxia; D = death; — = no clinical signs; MD = Marek's disease; MDV = Marek's disease virus; SPF = specific pathogen free; VE = virus exposed; pos = positive; neg = negative.

[B]Isolators each contained six chickens, two from each treatment. Check each treatment group for birds that shared same isolator.

[C]Inoculated at 81 wk with 2500 PFU by IA and SQ routes.

[D]Extra inoculated chicken added to replace donors that died early.

[E]Histologic examination not done.

[F]HVT vaccine inoculated at hatch; SB-1 and Md11/75C vaccines inoculated at 25 wk.

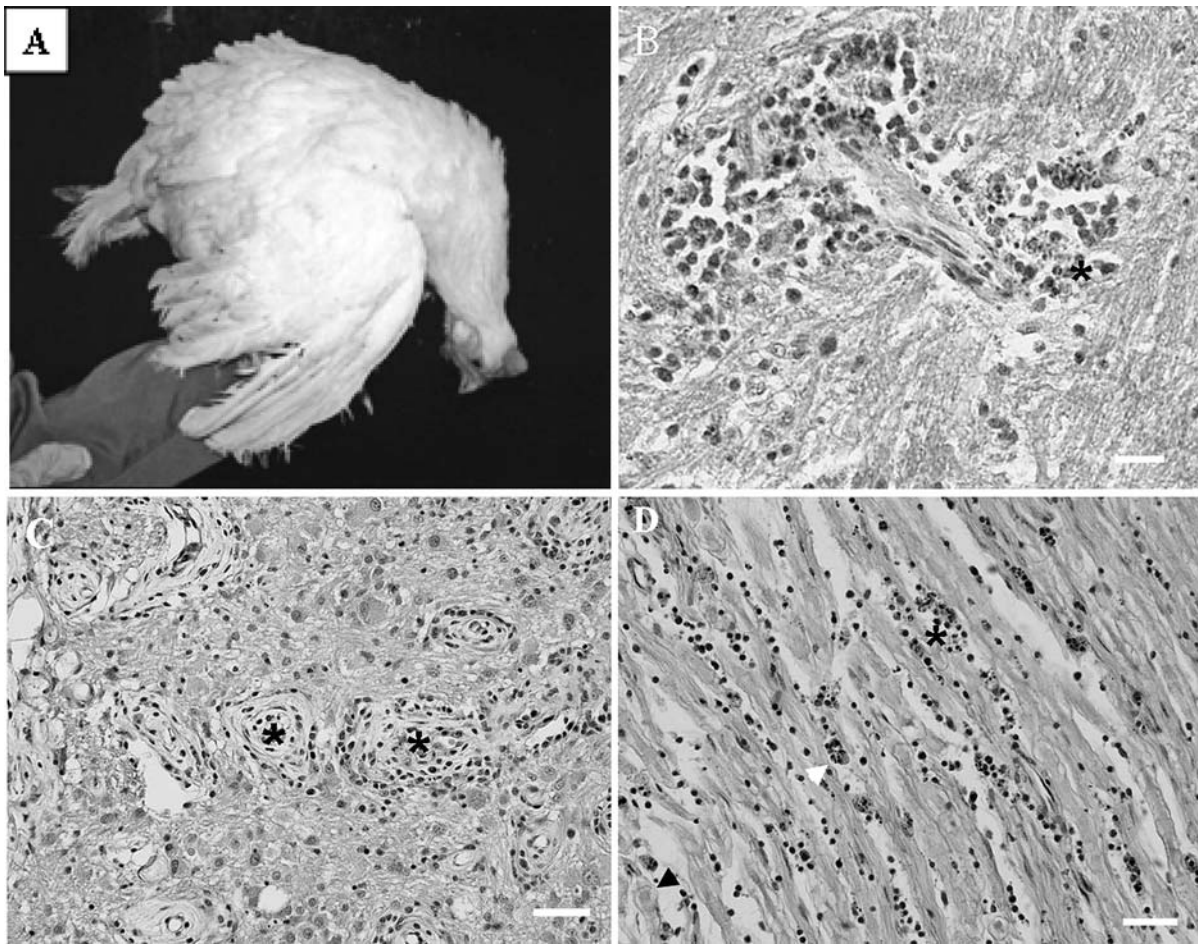

Fig. 1. Clinical TP and early lesions after inoculation of adult chickens with MDV (A) Clinical TP in a chicken at 9 days after challenge at 81 wk of age. Flaccid paralysis of the limbs and the neck can be observed. (B) Brain of a chicken 9 days after challenge at 30 wk of age. Severe vasculitis with extensive necrosis (asterisk), marked endotheliosis (arrow) and abundant infiltration of heterophils can be observed. Bar = 40 μm. (C) Brain of a chicken 11 days after challenge at 18 wk of age showing abundant vascularization and proliferation of endothelial cells around the blood vessels in the brain stem (asterisk). Bar = 100 μm. (D) Peripheral nerve of a chicken 9 days after challenge at 30 wk of age showing a severe neuritis with infiltration of lymphocytes, plasma cells, macrophages (arrowhead), and heterophils. Note the severe axon degeneration (arrow) and necrotic areas (asterisk) within infiltrates. Bar = 80 μm.

controls housed with VE controls in isolator 9 (Table 6). This indicated that the vaccine viruses administered to the VE chickens did not spread under the conditions of this trial. The 15 × 7 controls inoculated at hatch (housed in isolator HB6) developed 91% combined TP and MD, thus confirming the potency of the inoculum (Table 6).

Clinical and pathologic responses in adult chickens. Responses in adult SPF chickens were classed as early (9–15 DPI) or late (>28 DPI), which corresponds to the period of transient paralysis and MD tumors, respectively. The paucity of MD-related responses in adult VE chickens precluded description.

Early responses. The early response in adult SPF chickens was remarkably severe. Clinical TP in adult SPF chickens often had an acute onset, with flaccid paralysis of neck, wings, or legs (Fig. 1A), often followed by death in 1–3 days. The latent period to clinical signs was 1–2 days longer than in young chickens, but the disease was just as severe (Table 2). The frequency and lethality of the clinical TP syndrome appeared similar at different challenge ages, varying from 18 to 102 wk. Gross lesions were not observed in the peripheral nerves or other organs in the early response period. However, histologic lesions were detected in the brain and nerves as early as 8 DPI. Brain lesions were characterized by severe vasculitis with extensive necrosis of the vessel wall, fibrin deposition, and abundant infiltration of heterophils (Fig. 1B). Hyperplasia of endothelial cells was detected in all areas of the brain of infected chickens, but it was particularly remarkable in the brain stem, where up to eight concentric layers of endothelial cells could be observed around blood vessels. In three chickens inoculated at 18 wk of age, hyperplasia of endothelial lesions in the brain stem was very severe, infiltrating most parts of the neuropil and, in one case, extending into the meninges (Fig. 1C). Infiltration of lymphocytes, either as perivascular cuffs or diffuse in the neuropil, was moderate. Peripheral nerve lesions were surprisingly severe at early stages of infection, especially in chickens challenged at 30 or 81 wk. Affected nerves, although not grossly enlarged, often showed moderate infiltrations of lymphocytes, macrophages, plasma cells, and heterophils. In addition, varying degrees of edema, necrosis, and axon degeneration were commonly observed (Fig. 1D).

Late responses. The late responses documented in adult SPF 15 × 7 chickens that survived acute TP were generally unremarkable. Some birds that survived TP demonstrated a persistent ataxia (see Table 7), which is part of the persistent neurologic disease

syndrome (16). Of the 147 chickens in 21 groups that survived beyond 15 DPI after challenge at 18 wk with highly virulent MDV strains of vv+ and vv pathotypes (trials 2, 3, and 4), a high proportion developed typical gross lymphomatous lesions in nerves (65%) or viscera (51%; data not shown). However, of 20 chickens in five groups (trials 1A, 1B, and 5) challenged with highly virulent MDV strains at 30 to 102 wk and that survived beyond 15 DPI, eight (40%) had positive peripheral nerves, but gross enlargements were modest and frequently required histologic confirmation (data not shown). Visceral tumors were observed in two chickens (10%). Histologic lesions in enlarged peripheral nerves were of variable severity; some lesions were typical A-type, but others were more inflammatory in nature and included small lymphocytes, macrophages, and heterophils arranged in a perivascular distribution.

DISCUSSION

At least two alternative hypotheses explain outbreaks of MD in adult chickens. One theory is that adult populations are susceptible to new infection with highly virulent vv or vv+ MDV strains that cycle within adult populations and directly initiate the induction of tumors even at advanced flock ages (25, 30). This theory, later designated by Witter (48) as the new infection theory, implies that adult flocks of commercial-type chickens are susceptible to *de novo* infection (superinfection) with highly virulent strains despite existing levels of vaccine immunity and age resistance. An alternative theory, originally designated the old infection theory (48), is that late outbreaks are induced by MDV strains resident in the flock for varying periods of time that are triggered or exacerbated in adult flocks by as yet undiscovered environmental factors. The nomenclature ''old infection'' correctly implies that many late outbreaks could be from a virus infection acquired early in life but is misleading because the onset of disease is probably influenced more by environmental factors than by the age at MDV infection. Because commercial chickens are usually vaccinated and become infected early with MDV and because both vaccine and MDV challenge viruses persist for the life of the chicken, it is not easy to discriminate between these hypotheses.

The new infection theory can be tested by challenge of adult chickens with appropriate viral strains. In this model, commercial-type chickens would be expected to develop tumors at a significant rate subsequent to highly virulent challenge as adults. Although the old infection theory cannot be evaluated directly because the possible environmental factors are too numerous, it can be supported indirectly if commercial-type chickens develop few or no tumors subsequent to MDV challenge as adults.

These studies establish that adult SPF chickens at 18 to 102 wk of age are susceptible to challenge with highly virulent MDV strains. Data from 29 groups (trials 1–5) showed that such chickens developed a high frequency of clinical TP (often within 9 DPI) that often resulted in death (mean TP death = 44%). Chickens that survived beyond 15 DPI developed enlarged peripheral nerves or gross tumors at a high rate when inoculation was at 18 wk (mean of 24 groups = 72%) or at lower rates when inoculation was at 30 to 102 wk (mean of five groups = 40%). The total MD–related response rates were 83%–100% in susceptible lines (7_1, 7_2, and 15×7) and 60%–75% in three more resistant commercial strains. The mean MD-related response of 29 groups was 91%. Inoculated SPF chickens developed viremias and transmitted the challenge virus to contact birds. The seven vv+ or vv pathotype MDV strains all induced strong TP or MD responses, although the Md5 strain induced tumors but little or no TP. However, nine groups of adult SPF chickens challenged with four MDV strains representing the v pathotype (trials 1B and 3) were highly resistant to clinical TP (2.5%) or TP death (0%). MD responses ranged from 17% to 100% for three groups inoculated with the JM/102W strain and from 0% to 15% for six groups inoculated with three other v pathotype strains. The higher response to JM/102W in trial 3 (18-wk challenge) compared with trial 1B (102-wk challenge) might have been influenced by the age at virus exposure. TP induction by the different viral strains in adult chickens correlated well with previously established neuropathotype designations (15).

These observations are consistent with the brief report by Rosenberger *et al.* (37) on the susceptibility of adult SPF chickens to highly virulent strains. This is also supportive of the variable susceptibility of older SPF chickens to challenge with low virulence (v pathotype) strains (see introductory material). Thus, from these data, one could conclude that in the absence of protective immunity, adult chickens up to 102 wk of age have the capacity to develop strong TP and MD responses after high-virulence MDV challenge.

However, the primary objective of this study was to test the susceptibility of VE chickens to MDV exposure as adults. Ten lots of VE chickens were highly refractory to 18-wk challenge with seven highly virulent MDV strains of pathotypes vv and vv+ (trials 2 and 4). Of 187 VE chickens challenged, none developed TP and only a single bird in the Com-C group had MD lesions. The etiology of this single lesion is questionable and might have been induced by prior exposure to JM/102W at 5 wk rather than by challenge with 648A at 18 wk (see Results). The VE chickens were created by vaccination with HVT at hatch and exposure to the low-virulence JM/102W strain at 5 wk in an effort to simulate field exposure. However, stronger vaccines are typically used in commercial practice (e. g., bivalent or CVI988/Rispens), and the protective immunity induced in our trials might not necessarily be comparable to immunity in the field.

Studies of two groups of older VE chickens of the 7_1 strain revealed similar results (Table 6). One lot was inoculated at 81 wk and another was exposed by contact to inoculated, age-matched SPF chickens. No TP or MD was observed in either lot (16 birds total) although serotype 1 virus was isolated from single birds in each lot at 4 or 12 wk. The inoculated group appeared to transmit the virus as judged by the appearance of antibody in four of six SPF contact chickens. The VE chickens in this study were vaccinated with HVT (serotype 3) at hatch and then with SB-1 (serotype 2) and Md11/75C (attenuated serotype 1) at 20 wk, but there was no known exposure to virulent serotype 1 MDV before experimental challenge.

Thus, we conclude that VE chickens of highly susceptible genotypes or commercial crosses, when challenged as adults with highly virulent MDV strains, are highly refractory to the induction of TP and MD. Data from 203 birds in 12 groups showed that none developed clinical TP or TP death and only one died of MD (total MD–related response = 0.5%). In addition, these birds developed minimal virologic responses, and transmitted virus with difficulty. Superinfection of adult VE chickens with serotype 1 MDV did occur, at least in certain cases, but with minimal effect. However, this high resistance to infection and lesions might apply only to VE chickens with sufficient protective immune responses.

MD responses in adult SPF chickens were only modestly influenced by age, dose, or route of inoculation. Compared with challenge at 3 wk, challenge at 18 wk induced the same or greater total response and frequency of TP death. However, the TP responses of 3-wk chickens were lower in this trial than reported

previously (15), suggesting that the effect of age might be minimal. Strong MD-related responses with highly virulent MDV strains were induced in adult SPF chickens regardless of whether the inoculated dose was 10,000 PFU (90%) or 500 PFU (96%) or whether exposure was by contact (100%). Contact exposure induced a slightly higher rate of TP death than other doses, but in general, the three exposure types were not greatly different. Thus, adult SPF chickens were at least as susceptible to MDV challenge as young chickens and responded well to exposure by a variety of routes and methods.

The early pathologic responses in the brain and nerves of adult SPF chickens were remarkable compared with TP and MD responses in young chickens (exposed at 1 day or 3 wk), which have been well described elsewhere (13, 14, 16, 26, 32, 33) but were not studied in these trials. In the brain, infiltration of lymphocytes was less severe, but vasculitis with extensive necrosis of the vessel wall and endotheliosis were more severe than lesions reported in the brains of chickens infected at younger ages. The early pathologic response in the nerves also appeared to be more severe than in chickens inoculated at younger ages. In particular, the peripheral nerves of SPF chickens inoculated at 30 or 81 wk with vv+ strains, although rarely grossly enlarged, showed marked infiltration of lymphocytes, macrophages, plasma cells, and heterophils as well as edema, necrosis, and axon degeneration 9–10 days after virus challenge. In contrast, when SPF chickens were inoculated at hatch or 3 wk, histologic nerve lesions were absent or very mild at this same time regardless of whether the MDV strains were of v pathotype (6, 33) or the vv+ pathotype 648A strain (Gimeno and Witter, unpubl. data).

It is plausible that older SPF chickens with competent immune systems are able to elicit a strong immune response against new MDV infections. Such strong immune responses might be responsible for the severe damage in brain and nerves at early stages. Alternatively, cytolytic infections, macrophages, or cytokines could play a role in the development of these lesions (2, 21, 38), but these factors were not evaluated in this study. A systematic, controlled study is needed to better characterize the pathogenesis of MDV infection in older SPF chickens and elucidate the mechanisms involved.

The purpose of this work was to examine alternative hypotheses relating to the induction of MD outbreaks in older adult chickens. The new infection theory is that highly virulent MDV strains can cycle within adult populations and can directly initiate the induction of tumors even at advanced flock ages (25). This theory implies that adult flocks of MD-vaccinated commercial-type chickens are susceptible to superinfection with highly virulent strains, which break through existing levels of vaccine immunity and age resistance to induce lymphomas. However, the failure to induce significant MD lesion responses in 12 groups representing two types of VE chickens, all challenged with highly virulent vv or vv+ MDV strains, provides little support for this theory. Our studies do support the thesis that superinfection of VE chickens can occur and that chickens infected as adults can transmit the virus to pen mates, although the levels of response were extremely low.

One limitation of this study is that only two models of VE-type chickens were studied, both of which appeared to provide significant amounts of protective immunity at the several ages when chickens were challenged. In the field, the level of immunity or innate resistance of chickens can vary. If immunity is sufficient, we would predict that introduction of a new highly virulent strain to an adult chicken flock would not likely induce significant tumor mortality. On the other hand, if immunity in adult chickens is or becomes insufficient, then the probability for development of disease might increase. Our studies show that adult chickens, in the absence of prior MD-specific immune responses, can mount vigorous responses to MDV challenge.

Thus, we would predict that recent or contemporary introduction of highly virulent strains in commercial flocks would not be sufficient to induce clinical MD unless a proportion of the flock lacked sufficient protective immunity. MD protective immunity appears to last for long periods in laboratory studies (51). A lack of sufficient immunity at older ages in field flocks could be a failure of induction or a loss of immunity after induction. However, if immunity is not induced at a sufficient level subsequent to vaccination at hatch or *in ovo*, early MD losses would be expected. Therefore, outbreaks of MD in previously healthy adult chickens seems more likely related to compromised immunity, probably by environmental factors including stress and immunodepressive infections (30, 48). Chicken anemia virus has been frequently associated with MD outbreaks, including those in adult flocks (30, 38). Furthermore, chicken anemia virus can abolish cytotoxic T-cell responses to a second pathogen when both pathogens are simultaneously replicating (29, 38). It is unlikely that chicken anemia virus was a factor in our studies because virus stocks were clean and because adult VE chickens were resistant (whereas chicken anemia virus would be expected to increase susceptibility). Treatment with immunodepressive chemicals has also induced the onset of tumors in vaccinated and MD-exposed chickens (34).

An alternative hypothesis is that recrudescence of the thymus after molting (5) might favor the induction of lymphomas by increasing target cells. However, it is more likely that effects of molting on lymphoma induction, if any, might be mediated by a transient depression of immune responses (17, 18, 24). These effects in vaccinated birds might not always be sufficient to cause problems because molting did not abrogate immunity induced by a live attenuated *Salmonella* vaccine in adult hens (19). A better understanding of molting and stress on vaccine immunity to MD is needed.

Thus, it appears that the potential for late MD outbreaks in adult, MD-vaccinated flocks could be determined less by whether the infection is new or old than by other factors that influence the susceptibility of chickens to challenge. The nature of these factors is a matter of speculation and might differ among flocks. More work is needed to fully understand the mechanism of late MD outbreaks. In these studies, we were not able to reproduce an MD tumor outbreak in adult VE chickens. Until a model is developed for reproduction of the late MD outbreak syndrome in previously vaccinated chickens, elucidation of its etiology and pathogenesis will be problematic.

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